AD_____
(Leave blank)

Award Number:	W81XWH-10-2-0092
TITLE:	"Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress"
PRINCIPAL INVESTIGATOR:	Stephen Ahlers
CONTRACTING ORGANIZATION:	The Geneva Foundation Tacoma, WA 98402
REPORT DATE:	September 2013
TYPE OF REPORT:	Annual
PREPARED FOR:	U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

☒ Approved for public release; distribution unlimited

☐ Distribution limited to U.S. Government agencies only;
report contains proprietary information

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE September 2013	**2. REPORT TYPE** Annual	**3. DATES COVERED** *(From - To)* 1September2012-31August2013
Neurocognitive and Biomarker Evaluation of Combination mTBIfrom Blast Overpressure and Traumatic Stress		**5a. CONTRACT NUMBER** **5b. GRANT NUMBER** W81XWH-10-2-0092 **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) **Rayond Genovese, Ph.D., Jitandra Dave, Ph.D., and Stephen Ahlers Ph.D.**		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Geneva Foundation 917 Pacific Ave, Suite 600 Tacoma, WA 98402		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army MRMC Ft Detrick, MD 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of this research proposal is to systematically assess the combined effects of BOP and exposure to traumatic stress in rodents with the aim of understanding how these forces may interact with the manifestation of cognitive and emotive dysfunction, as well as evaluating their outcome on known biomarkers involved in TBI and stress response system activation

15. SUBJECT TERMS none provided

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		8	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION:

There is a high co-morbidity of mild traumatic brain injury (mTBI) and post traumatic stress disorder (PTSD) in Warfighters. Co-morbid mTBI and PTSD appears to be more prevalent than mTBI cases in isolation. Mild TBI and PTSD are statistically ranked the highest of battlefield injuries in OIF and OEF. It is generally assumed that the manifestation of mTBI symptoms result from one or more exposures to improvised explosive devices (IEDs) and that PTSD symptoms result from exposure to prolonged battlefield stress. The high incidence and comorbidity of PTSD and mTBI underscore an imperative for the DoD research community to gain an understanding of the underlying mechanisms that precipitate these conditions together with the often associated post-concussive syndrome (PCS) which appears to share many of the same cognitive and emotive symptoms associated with TBI and PTSD. The purpose of the proposed experiments is to determine the relative contributions of repeated exposure to blast overpressure (BOP) and exposure to stressful (predatory) events, when presented alone and in combination, in a rodent model. The level of BOP used in the proposed experiments has been demonstrated by the PI (Ahlers) to be associated with mild outcomes where there is evidence of cognitive impairment in the absence of demonstrable pathology. The proposed experiments take advantage of years of extensive experience from the primary investigators (Ahlers & Genovese) in studies of the effects of BOP and stressful events and their effects on behavior. The assessment behavioral outcomes resulting from exposure to BOP and stress will be complemented by the assessment of the potential protein biomarkers by Dr. Dave and his group who have considerable experience identifying protein biomarkers for brain injury.

BODY:

The objective of this research proposal is to systematically assess the combined effects of BOP and exposure to traumatic stress in rodents with the aim of understanding how these forces may interact with the manifestation of cognitive and emotive dysfunction, as well as evaluating their outcome on known biomarkers involved in TBI and stress response system activation.

Specific Aims

- Specific Aim 1: Assess the effects of repeated exposure to BOP and stress on cognitive and emotional performance. The primary investigator for this aim is Dr. Ahlers with support (predator exposures and performing the elevated plus maze) from Dr. Genovese.

- Specific Aim 2: To characterize the extent to which BOP will specifically modify the process of conditioned fear in rats. The primary investigator for this aim is Dr. Genovese with support (blast exposures) from Dr. Ahlers.

- Specific Aim 3: Evaluate the combined effects of repeated exposure to BOP and stress on established biomarkers of traumatic brain injury (TBI). Primary

investigator is Dr. Dave working in tandem with Drs. Genovese and Ahlers.Dr. Dave's effort is structurally aligned with Dr. Genovese's effort, as they are both WRAIR performers. Because of the technical sophistication of the experiments the overall plan emphasized the efforts of specific aim 2, which, as stated previously, has been completed. Note that the analysis of brain biomarkers relating to the exposure conditions from specific aim 2 are underway but not yet complete. Aim 1 to assess the acute effects of blast on cognition and anxiety is ongoing.

There are no changes to the original Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS: (Ahlers portion) We have completed the study to assess the effects of combined blast and stress exposures on spatial working memory using a Morris water maze. The results of this study did not demonstrate any differences on the acquisition of learning the spatial location for any of the experimental conditions leading to the conclusion that, at least within the parameters of the current study, the addition of a stressful experience to exposures to blast overpressure does not have an additive effect in impairing performance when the assessment occurs shortly after the exposures. As we have noted in previous published work, there is evidence that the effects of blast and stress do not appear to become manifest until some weeks or months after the exposures, particularly the blast exposure. We noted in previous reports that there is evidence for the cellular machinery reflecting brain damage to the prefrontal and cortical areas of the brain in rats exposed to 3 x 75 kPa BOP. Crucially, the evidence suggests that BOP exposure may increase markers of apoptosis, programmed cell death. Apoptosis processes and consequent cellular death and reorganizational processes make take a long period to take hold after blast, thus explaining why there are long-term effects of blast and stress but not short-term. We expect that the biomarker analysis on samples from rats exposed to blast in these studies will provide a clue to the potential for long-term outcomes.

REPORTABLE OUTCOMES:

For the first phase of the study the co-PI role was to provide animals exposed to blast overpressure and to assist in the preparation of the animals and tissue for the biomarker portion of the experiment. The major portion of the work beyond the above was the conditioned fear experiments performed by Gr. Genovese's laboratory. These data were reported in the report submitted by Dr. Genovese and will not be repeated here.

For the second phase of the study there are two experimental themes. In the first series of experiments, the assessment of combined blast and stress exposures on spatial cognitive performance using procedures and parameters that we have previously demonstrated impairment of performance with BOP exposure alone (Ahlers, et. al., 2012). The second series of experiments will assess the combined blast and stress exposures on measures of anxiety using the elevated plus maze. In this report we show the results of the assessment using the spatial working memory task. The assessment of anxiety is ongoing and will likely be reported in the first or second quarter of the next FY.

In this experiment we assessed the effects of repeated exposure to BOP and stress on cognition (spatial working memory) using the Morris Water Maze (MWM) task previously described as sensitive to detect cognitive impairment after exposure to 3 BOP exposures, one per day, on three consecutive days. Four treatment groups are proposed as defined in Table 1 below. For this experiment we utilized the BOP and MWM parameters previously described where slight impairment of acquisition was observed in four block trials after exposure to three BOP exposures (one per day under anesthesia) at the 75 kPa BOP intensity where rats are facing the blast wave inside the WRAIR shock tube. To assess the effects of explicit stress and BOP an additional group of rats were exposed to the BOP and to a different predator stress on three consecutive days. 24 hours after the behavioral tests animals will be euthanized and tissue samples will be taken for biomarker analysis. A summary of the experimental conditions is provided below:

Condition	n	Predator exposures	BOP	Dependent Measures	
				MWM	Biomarkers
Control-Sham	10	-	-	x	x
Control-Predator	10	x	-	x	x
Blast-Sham	10	-	x	x	x
Blast-Predator	10	x	x	x	x

In the basic 4 x 4 balanced experimental design shown above rats in the control (sham) condition were exposed to the BOP device without experiencing the blast and will also be exposed to a sham predator environment without explicit exposure to the predators. Behavioral assessment occurred 24 hours after the last exposure to BOP/stress or sham condition(s).

The results of the experiment are provided below. Figure 1 depicts the latency to find the sunken platform over the course of 4 blocked trials in which each block represented 4 trials. The four conditions outlined in the table above conditions are depicted. The solid black line depicts the control-sham condition. Over the course of the blocked trials rats in the control condition reached asymptotic performance by block three with only marginal improvement in latencies from block 3 to block 4.

There were no significant statistical differences between the four treatment conditions across the blocked trials despite the observation that the blast-predator condition appeared to acquire the task at a slower rate and to a lesser extent that the other treatment conditions. It may be that greater statistical power to distinguish the conditions may have been reached with additional animals. In our experience however, treatment effects with group sizes of 10 are not likely to manifest with higher numbers of animals per group. Additionally, based upon our prior experience and power calculations, 10 animals per group are adequate to see treatment effects using this paradigm. It is also noteworthy that there was not a distinct impairment in the blast group compared to the same condition, which represents a failure to replicate previous (unpublished) findings of a slight impairment of performance with this blast exposure regimen.

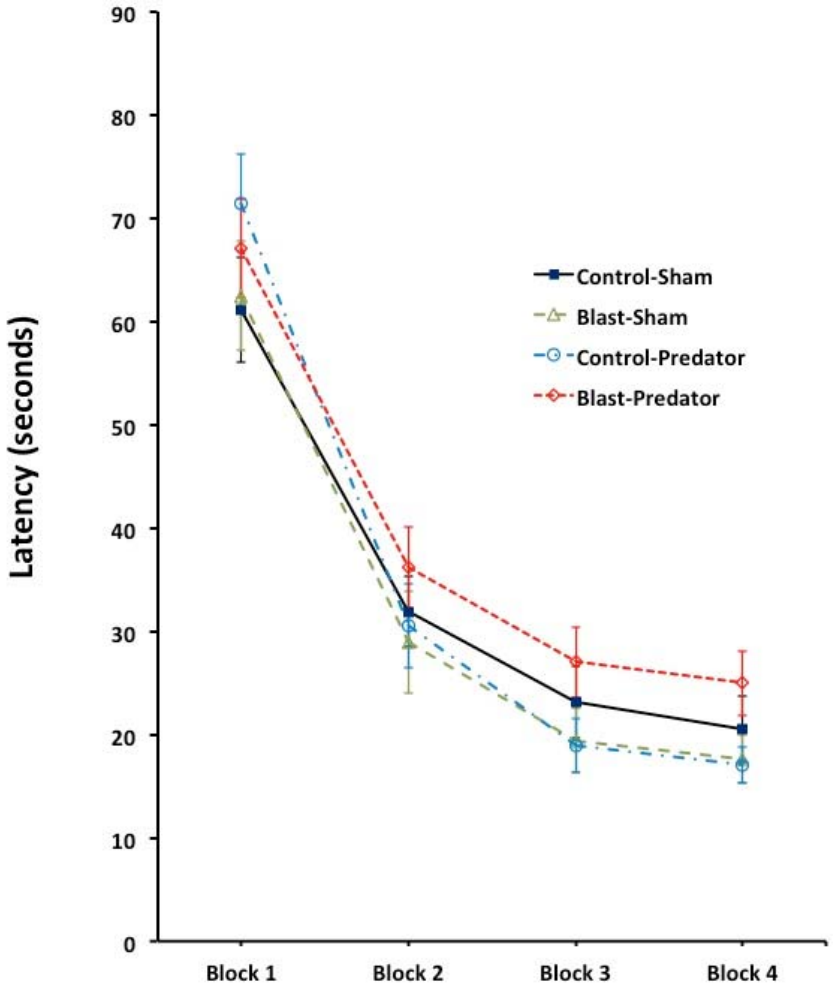

Figure 1. Effects of Repeated BOP and predator exposure on the Morris Water Maze: Mean latency time to find the submerged platform in four blocked trials presented in a single day.

CONCLUSIONS:

- The results of this study did not demonstrate any differences on the acquisition of learning the spatial location for any of the experimental conditions leading to the conclusion that, at least within the parameters of the current study, the addition of a stressful experience to exposures to blast overpressure does not have an additive effect in impairing performance when the assessment occurs shortly after the exposures.

- The project is on pace to complete the milestones provided in the proposal.

- The next portion of the study will focus on exposure of rats to BOP and to the stressful stimuli with the focus on the assessment of anxiety.

REFERENCES:

Elder, G.A., Dorr, N. P., De Gasperi, R., Gama Sosa, M. A., Shaughness, M. C., Maudlin-Jeronimo, E., Hall, A. A., McCarron, R. M., Ahlers, S. T. Blast Exposure Induces Post Traumatic Stress Disorder-Related Traits in a Rat Model of Mild Traumatic Brain Injury. *Journal of Neurotrauma*, 29:1-12, 2012

APPENDICES: None.

SUPPORTING DATA:

As part of another collaboration, we (Ahlers) recently published a paper examining the long-term effects of exposure to blast overpressure on the manifestation of anxiety behaviors several months after the blast exposure. The blast exposure parameters are similar to those employed in this study; however the studies are distinct in several ways. The primary emphasis of this effort is the near simultaneous exposure to blast and stress whereas the Elder et al. paper examined the effects of blast on anxiety behaviors 4 months or longer after the exposure to blast only (no stress exposures). The reference to the Elder paper is provided above.